From proteome to interactome: a mechanistic approach to MS biomarker discovery

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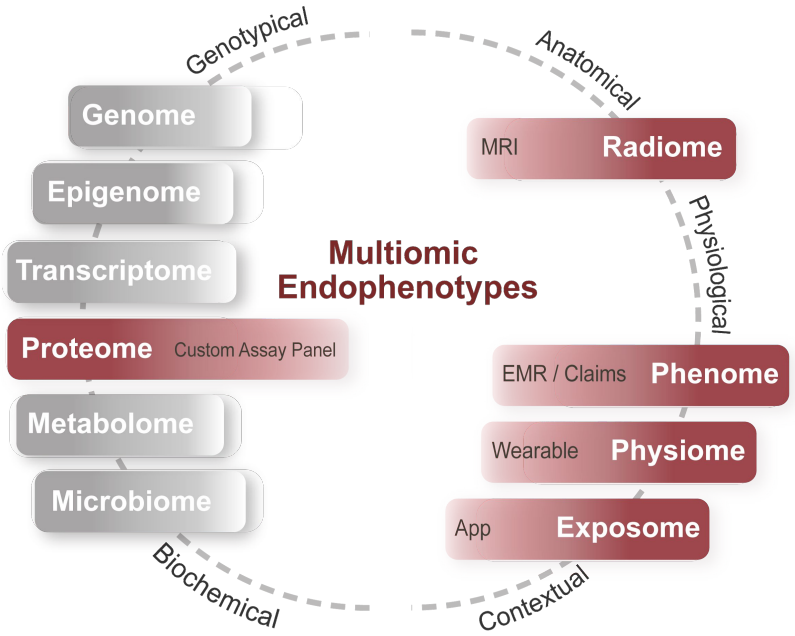
MS VIRTUAL 2020 8th JOINT ACTRIMS-ECTRIMS MEETING SEPTEMBER 11-13

ECTRIMS EUROPEAN COMMITTEE FOR TREATMENT AND RESEARCH IN MULTIPLE SCLEROSIS

Objective | Multi-Omic Integration of MS Measurements



Proteins → Pathways

- Reveal disease mechanisms-of-action
- Optimize drug selection
- Tailor therapeutic regimens
- Guide drug repurposing

Pathways + MRIs + Tracking + EMRs → Multidimensional Disease Profiles

- Provide 360° snapshot of disease state
- Augment granularity of patient health data
- Facilitate integrative disease assessment & management
- Refine MS subtypes for more precise stratification
- Inform patient-centered care delivery

1	MRI	Verification of Acquired Pathologic Phenotype + Monitoring of Its Evolution
2	Tracking	Real-time Periodic Logging of Physiology & Context
3	EMR	Incorporation of Outcomes + Offline Capture of Physiology & Context
4	**Proteins**	**Early Detection of Pathology + Direct Look Into Underlying Biology**

Disclosures:
Amal Katrib, Ferhan Qureshi, Michael Becich, and Victor Gehman are employees of Octave Bioscience.

Susan Goelz serves as the Scientific Director of the Myelin Repair Foundation. She has received consulting fees from Octave Bioscience for the design and planning of biomarker experimental validation studies.

Computational **PREDICTION** —*characterizing underlying biology*→ Signal **REPRODUCIBILITY + CLINICAL ACTION**

Disease Biology

- Complex etiology
- Integrated network of cells, tissues & organs
- Blood—"indirect" window into pathophysiology
- Binocular focus on neurofilament light (NfL)—a neurodegenerative marker
- Variable disease trajectory
- Inter-/Intra-patient heterogeneity

Multiple data streams +
Multiplex protein assay +
Functional annotation & mechanistic modeling +
Multi-layered systems integration

Study Design

- Predominantly qualitative endpoints
- Discrepancy in reported clinical / surrogate endpoints
- Underpowered single-cohort studies
- Sampling & retrospective analysis bias
- Insufficient MS population representation
- Scarcity of multimodal longitudinal measures

Multiple studies +
Field-established quantitative endpoints

Analytics

- Deficient quality control practices
- High quantitative assay variability
- Soluble marker intra- & inter-patient heterogeneity
- Setbacks in research to clinical-grade assay translation

Olink proteomics +
In-house lab operation +
Fit-for-purpose Assay Validation

Post-Analytics

- Limited employment of multivariate statistics
- Weak causality distinction
- Lacking / Incomplete control of confounders
- Poor validation of statistical findings
- Overlooked assessment of biological significance

Elaborate data science pipeline +
Biomedical knowledge graph +
Hypothesis testing & experimental validation

Materials and Methods

Input

Multivariate Analysis

Blood serum proteins predictive of disease activity **(n = 21)**

- **Per radiographic endpoints:**
  - Gadolinium-Enhanced Lesion Count; T2 Lesion Volume
- **Per clinical endpoints:**
  - Expanded Disability Status Scale (EDSS); Clinical Relapse Status; Annualized Relapse Rate (ARR)
- **Feasibility assessment using:**
  - Serum Pools + Matched Normals (n = 82)
- **Biomarker discovery using:**
  - ACP[1] + CLIMB[2] (n = 305)
- **Research & development using:**
  - CLIMB + EPIC[3] + Univ. Basel (n = 653)
- **Clinical validation using:**
  - SUMMIT[4] + RMMSC[5] (n ≈ 1000)

Analysis

Spatial Expression Profiling

- Protein biomarker relative quantification values correlated to:
  - **Human Protein Atlas** aggregated lymphoid tissue, peripheral blood immune cell type and brain region proteomic data
  - **Allen Brain Atlas** brain structure + cell type transcriptomic data

Protein-Protein Interaction (PPI) Modeling

- Biomarkers input into **STRING** for network construction
  - Using physically- and functionally-associated proteins that exhibit minimum interaction score of 0.7 ('high confidence')
  - **Markov Cluster (MCL)** algorithm implemented to detect distinct subgraphs of interconnected proteins
  - Undirected network imported in **Cytoscape** for topological surveillance and calculation of centrality metrics

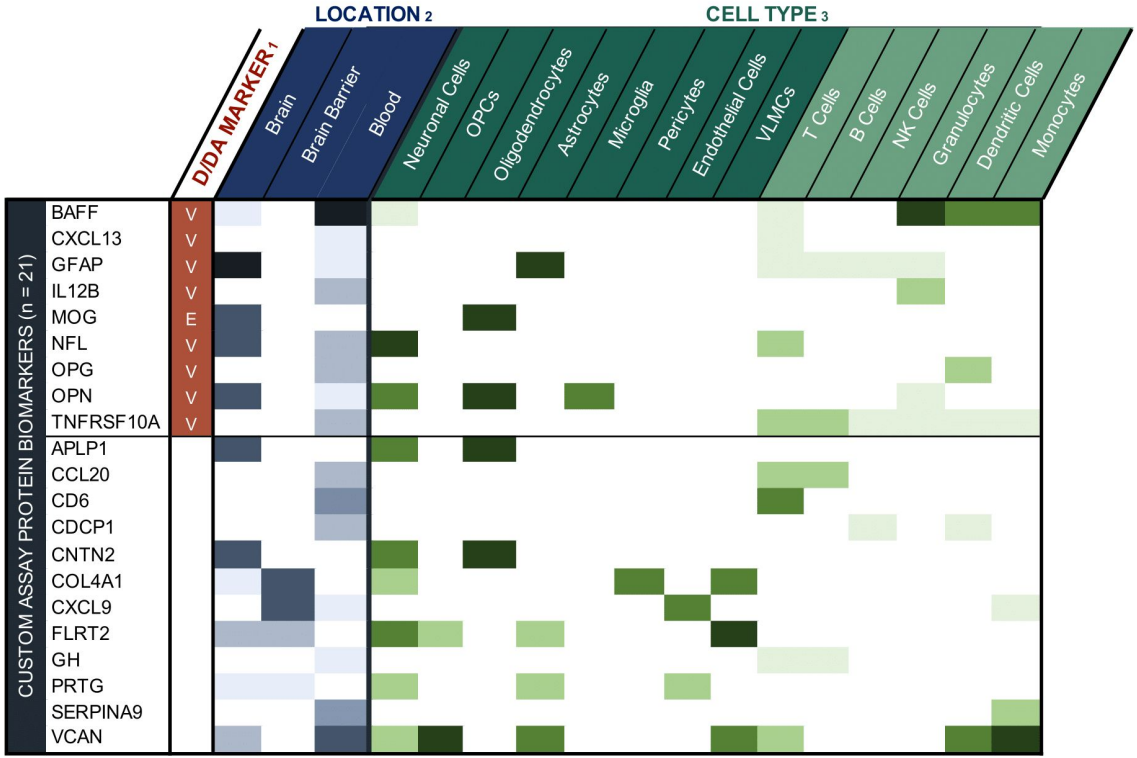
Gene Set Enrichment

- **Enrichr** leveraged to functionally annotate protein subgraphs
  - Significantly enriched terms extracted using z-score-adjusted Fisher's exact test p-value > 100

[1] Accelerated Cure Project
[2] Comprehensive Longitudinal Investigation of MS at Brigham and Women's Hospital
[3] Expression, Proteomics, Imaging, Clinical at UCSF
[4] Serially Unified Multicenter MS Investigation
[5] Rocky Mountain MS Center

Comparative proteomic analysis across human organs, tissues and cell types:

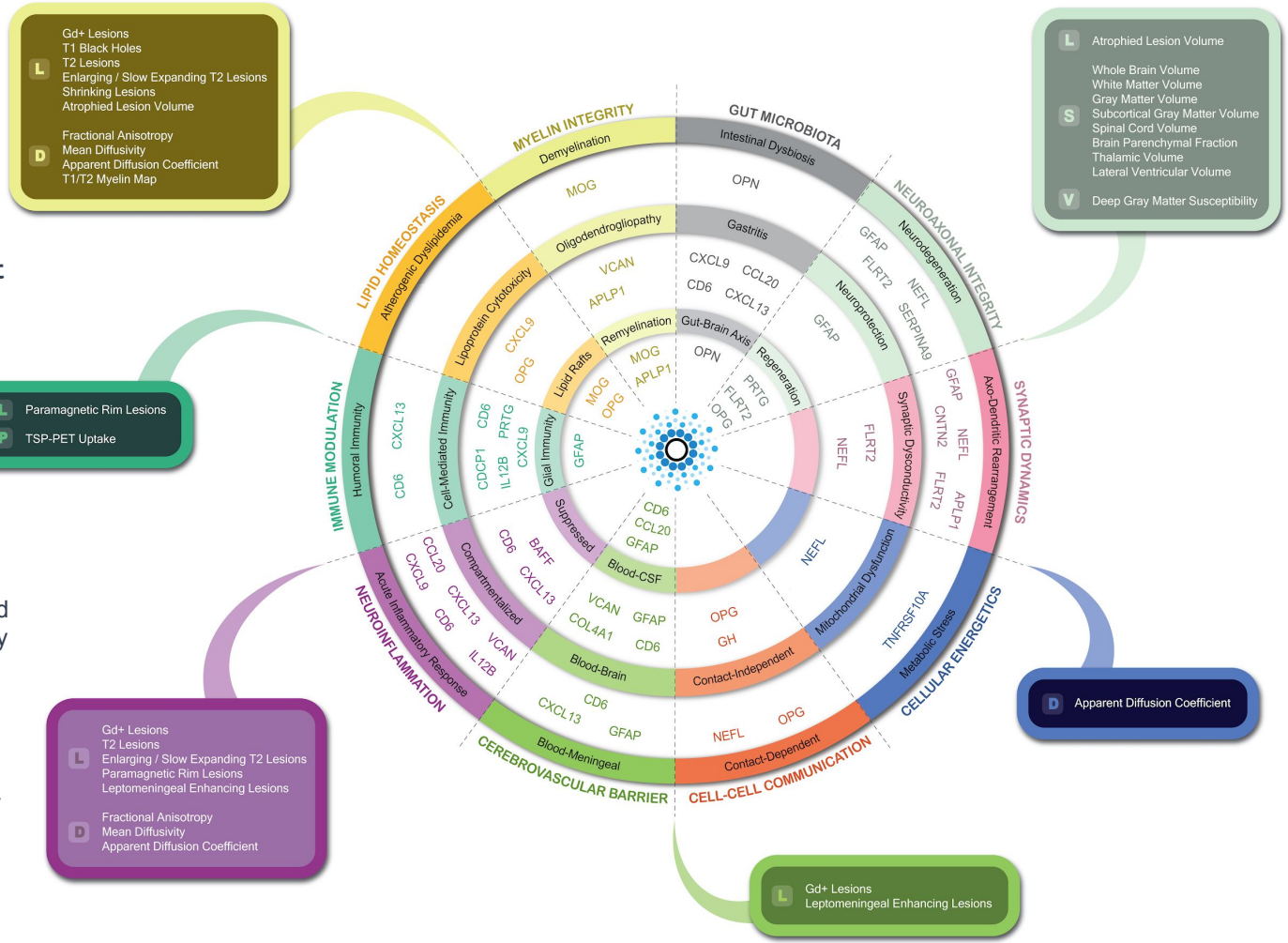
- helped hone in on MS-relevant information
  by isolating organ-specific blood analytes
  to address the blood's pervasive nature
- revealed the MS-specific locality
  of pre-selected protein biomarkers, to facilitate
  downstream assessment of directionality
- revealed a rich repertoire of MS cell types
  constitutively expressing these biomarkers,
  to facilitate downstream mechanistic modeling



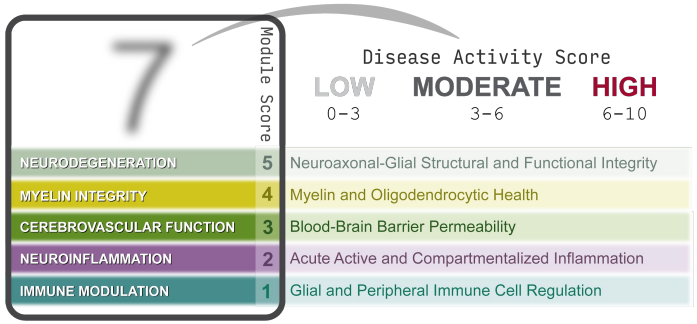
Results | Biomarker-Mediated Extrapolation of MS Disease Profiles

Network topological mapping of protein interactions and gene set enrichment analysis uncovered:

- an all-encompassing representation of MS hallmarks
  expanding beyond disease activity processes to inform worsening and progression
- the pleiotropic functionality of protein biomarkers in MS
  to isolate stage-specific adaptations and decode disease trajectory heterogeneity
- corresponding radiographic surrogate endpoints
  to later integrate proteomic findings with MRI features for a more in-depth characterization of MS pathophysiology



Results | Hypothesis-Mediated Interpretation of MS Biomarker Findings



Protein	Directionality (a) nonGd+ → Gd+ MRI Lesion Shifts	Directionality (b) nonGd+ vs Gd+ MRI Lesion Shifts & Cross-Sectional	Disease Activity Effect
NfL	4.01	5.22	Strong Positive
CXCL9	-3.39	-2.85	Strong Negative
CD6	-3.34	-2.05	Strong Negative
IL-12B	-3.14	-2.08	Strong Negative
BAFF	-2.87	-2.12	Strong Negative
CDCP1	-2.46	-2.47	Strong Negative
CCL20	-1.57	-2.12	Moderate Negative
OPG	-1.99	-1.66	Moderate Negative
TNFRSF10A	-1.74	-1.50	Moderate Negative
SERPINA9	-1.09	-1.52	Moderate Negative
CNTN2	-1.49	-1.09	Moderate Negative
PRTG	-1.55	-0.85	Moderate Negative
GFAP	1.20	1.20	Moderate Positive
MOG	0.31	1.86	Moderate Positive
GH	0.89	0.89	Weak Positive
FLRT2	-1.32	-0.16	Weak Negative
APLP1	-1.24	0.17	Weak Negative
OPN	0.23	0.66	Weak Positive
CXCL13	-0.31	-0.36	Weak Negative
COL4A1	-0.26	-0.02	Weak Negative
VCAN	-0.45	0.51	Weak

- Directionality scores capture differential protein expression measures for Gd+ vs nonGd+ MRI lesion presentation (both longitudinal shifts & cross-sectional differences), weighted by study sample size via Stouffer's Z-score method
- Mechanistic hypotheses are provided for top 4 protein biomarkers with the highest overall directionality-informed disease activity effect

NfL

FORWARD DIRECTIONALITY

- Association with MS Disease Activity is well-established and highly reported

CXCL9

Conceptualized Pathophysiology

NEUROINFLAMMATION:

- Acute Inflammatory Response → CNS Extravasation (Diapedesis) of Mononuclear Inflammatory Cells
- Acute Inflammatory Response → Proinflammatory Cytokine & Chemokine Secretion

2 The IFN-γ-inducible CXCR3 chemokine ligand—constitutively expressed on microvascular endothelial & glial cells—is pivotal to the transendothelial migration & selective extravasation of effector lymphocytes into tissue injury sites. It drives a strong Th1 immune response that, in turn, triggers a self-perpetuating & propagating positive feedback loop to support CNS retention of autoreactive T cells & sustain local inflammatory cascade.

IMMUNE MODULATION:

- Leukocytic Cell-Mediated Immunity → $CD4^+$ Th1/Th17 & $CD8^+$ Effector T Cell Activation & Differentiation

1 With pleiotropic functionality, it promotes $CD4^+$ T cell polarization & differentiation into effector Th1/Th17 cells.

LIPID HOMEOSTASIS:

- Lipoprotein Cytotoxicity→ Circulating LDL Transcytosis into Brain Capillary Endothelial Cells

GUT MICROBIOTA:

- Gastritis → Gastrointestinal Tract Inflammation and Mucositis

Disease Activity Signal Hypothesis

INVERSE DIRECTIONALITY

- CXCL9 CSF levels are frequently observed to positively correlate with MS disease activity. This observation, however, dwindles when using blood serum measures that exhibit a less significant association with radiographic presentation and severity outcomes.
- We posit that the biomarker's high CSF-to-serum ratio may indicate a predominantly intrathecal synthesis (glial) and inflammation source (compartmentalized) for patients with high CXCL9 CSF measures.
- Such effect is more characteristic of chronic than acute inflammatory activity, which instead corresponds to an intact blood-brain barrier and thus a lack of gadolinium contrast enhancement for those patients.

Results | Hypothesis-Mediated Interpretation of MS Biomarker Findings

CD6

Conceptualized Pathophysiology

CEREBROVASCULAR INTERFACE:

- Blood-Brain/Meningeal/CSF Barrier ⟶ Microvascular Endothelial/Arachnoid/Choroidal Tight Junction Reorganization

3 The lymphocytic surface receptor can heterotypically bind, with high affinity & stability, to the CNS barrier-rich adhesion molecule ALCAM, inducing tight junction remodeling & cytoskeletal rearrangement to compromise barrier integrity.

NEUROINFLAMMATION:

- Acute Inflammatory Response ⟶ CNS Extravasation (Diapedesis) of Mononuclear Inflammatory Cells

2 During the early-stage acute phase, this can promote the paracellular transmigration of peripheral leukocytes —namely IL-17-producing γδ T Cells. The evolving inflammatory milieu can then enhance the lipid raft clustering of ALCAM and its immunological synapse-like colocalization with CD6, thereby favoring the selective caveolae-mediated transcytosis of autoreactive lymphocytes—namely IL-2- / IFN-γ-producing $CD4^+$ αβ T helper 1 cells.

IMMUNE MODULATION:

- Leukocytic Cell-Mediated Immunity ⟶ $CD4^+$ Th1/Th17 & $CD8^+$ Effector T Cell Activation & Differentiation
- Leukocytic Cell-Mediated Immunity ⟶ Altered Negative Thymic Selection & Autoreactive Clonal Expansion
- Humoral Immunity ⟶ Autoreactive B1 Cell Activation & Self-Renewal

1 By colocalizing with the TCR/CD3 complex at the central supramolecular activation cluster, the ligand-receptor pair can also directly mediate the antigen-specific activation and polarization of T lymphocytes.

CELL-CELL COMMUNICATION:

- Contact-Dependent Interactions ⟶ Paracellular & Transcellular Vesicular Transport Modulation

GUT MICROBIOTA:

- Gastritis ⟶ Intestinal Epithelial Barrier Hyperpermeability

Disease Activity Signal Hypothesis

INVERSE DIRECTIONALITY

- Albeit unproven, it is speculated that late events in T cell activation downregulate CD6 surface expression—through proteolytic cleavage and ubiquitination—to homeostatically control ongoing immune response.
- This contributes to increased serum levels of soluble CD6, which may then enact the role of a “decoy receptor” to block the ligand interactions necessary for lymphocytic activation and CNS infiltration.
- This may explain the biomarker’s pronounced serum overexpression during subacute/chronic activity and recovery.

Biomarker measurements can, therefore, be leveraged to inform:

Activity State Transitions:
Early Preactive *(very low)* ⇄ Acute Active ⇄
Subacute Active ⇄ Chronic Active *(very high)*

IL-12B

Conceptualized Pathophysiology

IMMUNE MODULATION:

- Leukocytic Cell-Mediated Immunity ⟶ $CD4^+$ Th1/Th17 & $CD8^+$ Effector T Cell Activation & Differentiation
- Leukocytic Cell-Mediated Immunity ⟶ Natural Killer (NK) & Dendritic Cell Activation

1 Making up the p40 subunit of IL-12 heterodimeric immunoregulatory cytokine, it triggers the differentiation of naive $CD4^+$ T cells into IFN-γ-producing effector/memory Th1 cells & enhances the proliferation & cytotoxic activity of $CD8^+$ αβ T/NK cells during an immune challenge & following ligand-receptor binding.

NEUROINFLAMMATION:

- Acute Inflammatory Response ⟶ Proinflammatory Cytokine & Chemokine Secretion

Disease Activity Signal Hypothesis

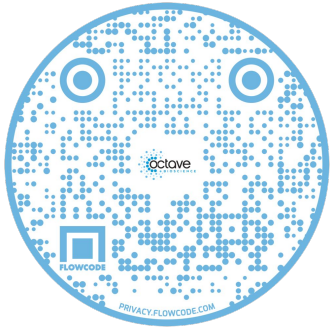
INVERSE DIRECTIONALITY

- With no reported significant changes in bioactive heterodimer IL-12 serum levels, we postulate that the homodimeric form (IL-12p40) may act as a competitive suppressant, blocking IL-12p70 ligand-receptor interaction & thereby antagonizing lymphocytic activation & selectively inhibiting IFN-γ production.
- We therefore anticipate a pronounced reduction in circulating IL-12B levels along with unperturbed or elevated heterodimeric IL-12 measures during acute inflammatory activity in MS.

Summary and Future Directions

- Through complementary integration of data analytics and systems biology, we were able to:
  - Biologically interpret multivariate classification findings
  - Augment prognostic capacity of blood-based predictive markers
  - Contextualize protein biomarker mechanisms-of-action with respect to MS disease activity, worsening and progression
  - Reveal protein biomarkers' ability to recapitulate full spectrum of MS pathophysiology
  - Unveil the orchestrated crosstalk between various molecular facets of MS
  - Switch MS biomarker discovery focus from single proteins *('protein space')* to protein disease dynamics *('network space')*

- In progress and next steps:
  - Experimental testing of protein biomarker mechanistic hypotheses and validation of directionality shifts with respect to disease stage, treatment course and overall patient health status
  - Weighted protein co-expression network analysis using absolute quantification measurements
  - Protein subnetwork correlation with clinical phenotypes for further multi-layering of MS data
  - Drug target enrichment analysis to inform DMT selection and drug repurposing
  - Analytical and Clinical Validation of 21-plex Custom Assay Panel



Questions? Contact akatrib@octavebio.com

Additional Posters P0055 • P0063 • P0091 • P0552 • P0583 • P0590